DEVELOPMENT OF PREFERRED ORIENTATION IN THE EGGSHELL OF THE DOMESTIC FOWL

R. M. SHARP
Department of Chemical and Materials Engineering, University of Auckland, Auckland, New Zealand

H. SILYN-ROBERTS
Department of Zoology, University of Auckland, Auckland, New Zealand

ABSTRACT Preferred orientation in the shell of the domestic fowl is shown by x-ray diffractometry to develop gradually throughout the shell, beginning immediately after the start of shell deposition and reaching a maximum at the exterior surface. Only 2 out of 20 shells examined exhibited a single preferred orientation: This was one in which the pole of the (001) plane lies parallel to the shell surface normal. The remaining shells had two preferred orientations present simultaneously, one in which the (001) pole is parallel and the other in which the (104) pole is parallel to the surface normal. Previous work has resulted in conclusions that are in conflict with these; they are discussed in relation to the present work.

INTRODUCTION

The orientation of the calcite crystals making up the three morphologically distinct regions characteristic of the eggshell of the chicken (*Gallus domesticus*), namely the cone, central, and outer layers, has been the subject of a number of studies. X-ray diffraction has shown that the calcite at the exterior surface of the shell shows some degree of preferred orientation. Cain and Heyn (1964) determined that the normal to the (001) plane of the hexagonal calcite lattice was oriented at an angle of 28 ± 16° to the shell normal. On the other hand, Favejee et al. (1965) found that the normal to the (104) planes was rigorously parallel to the shell normal. In contrast to this, Terepka's (1963) polarized light microscopy of thin sections suggested that the *c*-axes were primarily oriented perpendicular to the surface of the shell. Using electron diffraction from single crystallites, Perrott et al. (1981) concluded from chemically-thinned specimens that the central layer showed essentially random orientation. However, in an ion-beam thinned specimen, the same authors detected a small degree of preferred orientation within a single column, such that the *c*-axis lay within a range of 30 ± 18° to the surface normal. Results from different columns showed no preferred orientation within the central layer as a whole.

It has, therefore, been held that only the outer layer, the final stage of calcite deposition, exhibits any form of texture and that during shell deposition it is laid down onto central layer, which is believed to be, like the cone layer, composed of randomly oriented crystallites. In view of the known characteristics of crystal growth in poly-crystalline structures such as metal castings, it is to be expected that a preferred orientation manifested at the final stage of a structure that has formed by sequential crystallization will be the result of an intensifying development of that orientation throughout the material. Indeed, no obvious mechanism exists for the deposition of oriented crystallites directly on to the surface of a random crystalline array.

This study was designed to establish whether there is any development of preferred orientation during shell deposition and, using x-ray diffractometry, to resolve the difference in results obtained by previous workers for the final texture of the outer layer of the chicken shell. The technique used involves measuring the intensities of peaks associated with the different planes of the calcite lattice, at successive stages through the thickness of the shell. The changes in intensity can then be interpreted in terms of a preferred orientation, using the known interplanar angles of the calcite lattice.

SPECIMEN PREPARATION

All shell segments used were taken from the equatorial region of shells from unmated birds and were cleaned of membrane by boiling for 5 min in 5% (wt/vol) sodium hydroxide. Each segment was attached to a steel stub of 20 mm diam and 3 mm thickness using quick-curing epoxy resin. The segment, large enough to cover the whole area of the stub, was manually flattened on to it and then hydraulically flattened under a pressure of 20 MPa for 5 s. This process can be shown by scanning electron microscopy to cause no crushing of the shell; it results in an effective flattening of the segment by fine fragmentation enabling an accurate progression of grinding stages to be achieved. If shell segments are merely flattened manually, then each small fragment of shell has a degree of curvature; this results in different parts of a fragment's thickness being exposed at each grinding stage.

Starting at the interior surface of the shell, the segment having been attached by its exterior face, successive stages through the shell were exposed by manually grinding the surface using wet 1,200 grit silicon carbide paper placed on a glass plate. Grinding was done to a tolerance

of ±5 μm over the whole area of the stub and was monitored by measuring, at 10 points, the thickness of both stub and shell using a micrometer graduated in 10 μm intervals. Since the stub was of constant thickness and the flattening method used resulted in a shell that was plane within ± 5 μm, this technique, when accurately executed, allows the exposure of successive stages through the shell at very finely graduated intervals. Chicken shells are ~350 μm thick and an interval of ~50 μm was selected between each grinding stage.

The penetration of x-ray radiation into the shell at the highest Bragg angle used in this study can be shown (see Appendix) to be ~40 μm. Samples <50 μm thickness were therefore not analyzed. Results for the exterior surface were obtained from a contiguous equatorial fragment, mounted as before but with the exterior surface uppermost. To check the validity of the method and to establish whether interference from deeper layers affected relative peak intensities at any stage, two contiguous segments from the equator of a shell were analyzed, one by grinding successively from the interior surface and one from the exterior. Identical profiles were obtained.

METHOD OF ANALYSIS

Using a Philips diffractometer (Norelco; Philips Nederland BV, Eindhoven, The Netherlands), x-ray diffraction spectra were obtained from the interior and exterior surfaces of shell segments and from successive stages through the thickness of the shell. At each stage, the stub, with the attached shell, was fitted into the standard holder of the diffractometer and accurately adjusted for height. Filtered CoKα radiation was used and the specimen was rotated during analysis. Intensity ratios (I/I_0) of the peaks corresponding to the (102), (104), (006), (110), (113), (202), (108), and (116) planes of the calcite hexagonal unit cell were obtained by comparing, for each plane, its peak height above background (I) with that obtained from a random standard specimen (I_0). The random specimen consisted of chicken shell, cleaned of membrane by boiling for 5 min in 5% (wt/vol) sodium hydroxide, and then ground to pass through a 45 μm mesh. The resulting fine powder was placed in the standard powder holder of the diffractometer and the surface rendered flat without pressure on the powder. (Pressure appears to induce a degree of preferred orientation at the surface by an alignment of the cleavage planes of the individual grains). Peak heights for the random specimens were repeatable between five different standard specimens to within ± 4% and were consistent with those obtained from the interior surfaces of all shells, where the calcite is also oriented randomly. Table I lists the intensities measured for all the main peaks expressed as a percentage of that of the (104) peak and compares them with those obtained by Swanson and Fuyat (1953), which are reproduced in the JCPDS Powder Diffraction File.

TABLE I
PEAK HEIGHTS FOR THE MEAN OF FIVE RANDOM STANDARD SAMPLES COMPARED WITH THOSE IN THE JCPDS POWER DIFFRACTION FILE

	Peak heights*	
hkl	Measured	JCPDS file No. 5-586
(102)	8	12
(104)	100	100
(006)	2	3
(110)	11	14
(113)	17	18
(202)	15	18
(108)	15	17
(116)	16	17

*Peak heights are expressed as a percentage of that of the (104) plane.

The angles between the lattice planes were calculated according to the formula for interplanar angles in a hexagonal crystal system, viz

$$\cos\theta = \frac{h_1h_2 + k_1k_2 + (h_1k_2 + h_2k_1) + \frac{3}{4}\frac{a^2}{c^2}l_1l_2}{\sqrt{\left(h_1^2 + k_1^2 + h_1k_1 + \frac{3}{4}\frac{a^2}{c^2}l_1^2\right)\times\left(h_2^2 + k_2^2 + h_2k_2 + \frac{3}{4}\frac{a^2}{c^2}l_2^2\right)}}$$

where $h_1k_1l_1$ and $h_2k_2l_2$ are the Miller indices of the two planes, and a and c, for the calcite unit cell, are 4.959 and 17.060 Å, respectively (Winchell, 1956).

The relationship between the planes is best understood in terms of standard projections for the calcite lattice. We show here (Fig. 1) portions of two standard projections, based on the (104) and (001) planes. It is likely that any preferred orientation is based on the growth direction (normal to the plane of the shell) and, furthermore, it is likely (as Favejee et al. [1965] have found) that there will be rotational symmetry around this direction. In the experiments carried out here, the specimens were rotated around an axis perpendicular to the shell's surface, so that rotational symmetry will always be observed. A preferred orientation can then be represented as an inverse pole figure by radial contour lines on the appropriate standard stereographic projection, the spacing of which represent the degree of preferred orientation. A shell that has, for example, perfect (104) preferred orientation will have all its crystallites oriented so that their (104) planes lie in the plane of the shell's surface and will show only a (104) reflection. If the preferred orientation is less than perfect, then there will be a high peak corresponding to the (104) reflection and lesser peaks corresponding to other reflections, the intensity ratios (I/I_0) of which will decrease as the angle subtended with the (104) plane increases. Plotting I/I_0 values against angle subtended to a particular plane, here called the reference plane, will give a smooth curve if that reference plane is the plane of preferred orientation. The slope of the

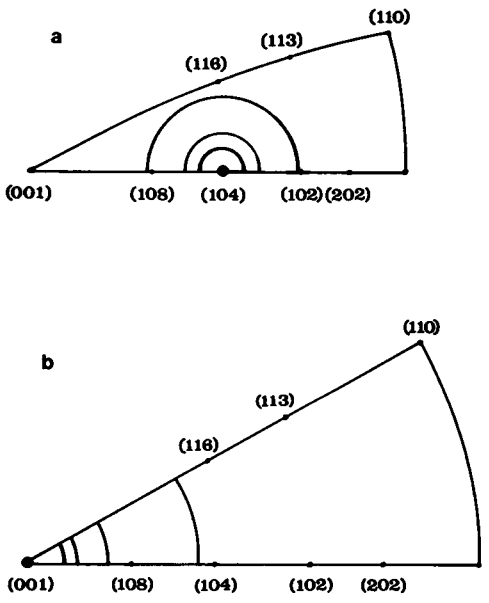

FIGURE 1 Portions of standard projections for the calcite lattice, based on (*a*) the (104) plane, (*b*) the (001) plane.

curve gives a measure of how well developed the preferred orientation is. By contrast, a randomly oriented specimen will obviously have I/I_0 values which are invariant and equal to unity for all planes.

RESULTS

Of 20 shells examined, the exterior surfaces of only 2 showed peak intensities consistent with the presence of a single preferred orientation. Results plotted using the (001) plane as reference showed the smooth curve indicative of a preferred orientation, while none of the other lattice planes when used as reference plane produced similar results. The preferred orientation at the exterior surface of these two shells was therefore one in which the pole of the (001) plane, i.e., the *c*-axis, lies parallel to the shell surface normal.

The development of this texture was established by analysis of the shell showing the higher (006) intensity ratio. At the initiation of calcite deposition, as represented by the tips of the cones, the orientation of the crystals was random, in that the values of the intensity ratios (I/I_0) for all planes were close to unity. Development towards the (001) preferred orientation was evident at only 30 μm into the cone layer (Fig. 2). Thereafter, the increasing slope of the curves for each successive stage through the shell indicated the presence of a gradually increasing intensity of the preferred orientation. Its maximum intensity was at the exterior surface of the shell but was, however, at an I/I_0 value of 5.25, very weak in comparison with the (001) textures developed in the ratite and tinamou eggshells (H. Silyn-Roberts and R. M. Sharp, 1984). Analysis of the whole equatorial region of the shell and of the two poles showed the presence all over of the single (001) texture. The maximum intensity ratio varied little on the equator but showed values of 0.52 and 0.38 times the equatorial value at the blunt and sharp poles, respectively.

Results from the exterior surfaces of the other 18 shells, plotted so that the (001) plane was the plane of preferred orientation, showed curves that were not consistent with there being only an (001) preferred orientation in that they had two maxima, one at the (006) position and the other at the (104). The heights of these maxima were variable; that of the (006) was greater in all but four of the shells, while that of the (104) in six of the shells was discernable only as a slight rise in the otherwise smooth curve of the (001) texture. Maximum values of I/I_0 for the (006) plane ranged from 2.0–7.0, with a mean value of 3.96 (SE = 0.29), and that for the (104) from 1.47–5.2, with a mean of 2.73 (SE = 0.24). No shell gave results that were indicative of a single (104) preferred orientation.

The double texture shell exhibiting the greatest intensity ratios of the (006) and (104) planes was analyzed to determine how the texture developed through its thickness. Over the first half of its development (Fig. 3), it was similar [albeit with slightly higher values for the (104) plane] to that seen in the shell having the single texture.

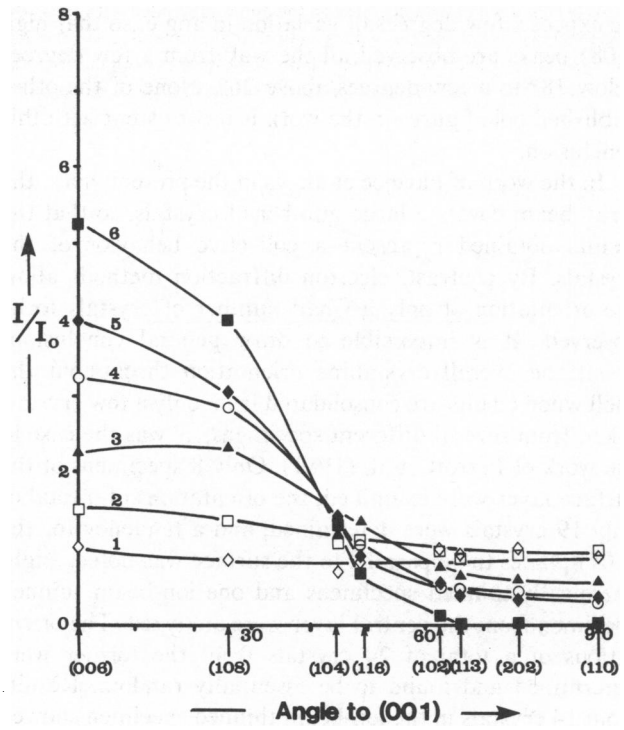

FIGURE 2 Development of preferred orientation in shell showing single texture. Stages: *1*, interior surface; *2*, 30 μm from interior; *3*, 80 μm; *4*, 130–180 μm; *5*, 230–270 μm; *6*, exterior surface. Shell thickness, 320 μm.

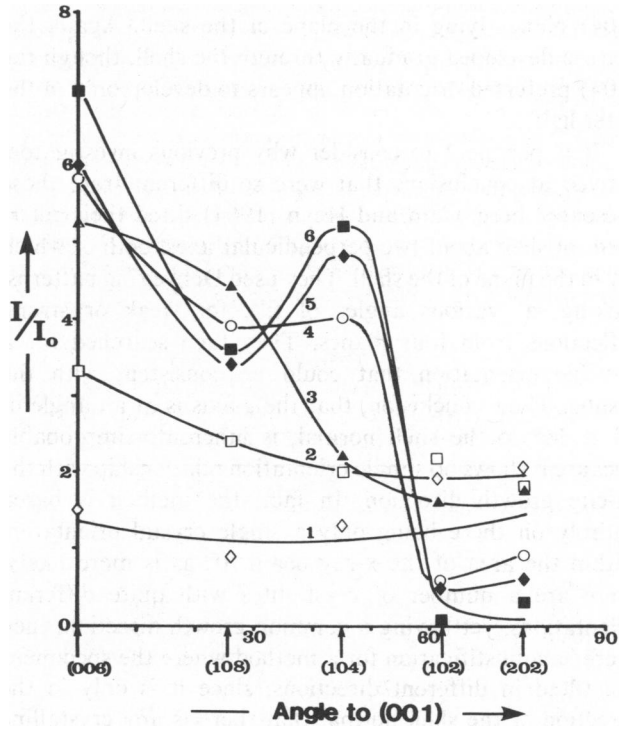

FIGURE 3 Development of preferred orientation in shell showing double texture. Stages: *1*, interior surface; *2*, 45 μm from interior; *3*, 95–145 μm; *4*, 195 μm; *5*, 245–295 μm; *6*, exterior surface. Shell thickness, 370 μm.

The initial development was thus towards that in which the (001) was the plane of preferred orientation. However, at 195 μm from the interior, almost exactly halfway through the shell, there was an appearance of a simultaneous (104) texture, as shown by the slight discontinuity in the smooth curve for that stage. Thereafter, developing simultaneously with an increasing (001) orientation was that of an intensifying (104), both reaching their maxima at the exterior surface. This double texture was present over the entire surface of the shell and the intensity ratios of both the (006) and the (104) planes showed little variation around the equator or along its longitudinal axis.

Note that when plotting peak intensity ratios for the shells having a double texture, those associated with the (110), the (113), and the (116) planes cannot be included, as they can be for a single texture (Fig. 2) because, in the double texture case, the contours on the standard (001) triangles are not arcs of circles. In fact, the smooth curve that can be drawn through points from all the planes in the single texture case is good indirect evidence for the absence of a double texture in those few shells that do not possess it.

DISCUSSION

Of the shells examined, a small proportion showed a single, fairly weak (001) preferred orientation that developed gradually from a randomly oriented crystal structure at the interior surface. The rest of the shells had a double texture at the exterior surface with a preference for both (001) and (104) planes lying in the plane of the shell. Again, the texture developed gradually through the shell, though the (104) preferred orientation appears to develop only in the outer half.

It is pertinent to consider why previous investigators arrived at conclusions that were so different from those presented here. Cain and Heyn (1964) tilted their specimens of shell about two perpendicular axes, both of which lay in the plane of the shell. They used Debye ring patterns, looking, at various angles of tilt, for weak or strong reflections from four planes. They then searched for a possible orientation that could be consistent with the results. Their conclusion, that the *c*-axis is at an angle of 28 $\pm$ 16° to the shell normal, is inherently improbable because it shows no simple orientation relationship with the calcite growth direction. In fact, the method is based entirely on there being only a single crystal orientation within the area of the x-ray beam. If, as is more likely, there are a number of crystallites with quite different orientations, yet having a common growth direction, then there is no justification for a method where the specimens are tilted in different directions, since it is only in the direction of the shell normal that there is any crystalline symmetry. There can, in any case, be no validity for consolidating all the data from several different crystallites into a single consistent orientation. Another serious criticism of the method is that moving the beam from place to place on the specimen may cause it to cover a new set of crystallites that have different orientations. This has obviously been the case here, as the authors themselves point out, because the intensities from the series I and series II experiments, where there is 0° tilt to the axes, ought to be identical but are, in reality, quite different. In these circumstances, the consolidation of data into one orientation is clearly not possible and the result has no validity.

The approach of Favejee et al. (1965) was more logical in that they used a texture goniometer to obtain the result that the (104) planes were parallel to the shell surface. The possibility of a double texture was not considered by them and would not have revealed itself because they did not use the (006) peak at all in their experiments. The authors of this work did however note, but offered no explanation for, the fact that the (108) reflection had its highest intensity at a position where the normal to those planes would be at an angle of ~30° to the shell normal. They pointed out that this was inconsistent with the fact that the interplanar angle between the (104) and (108) planes is only 18°. Careful examination of their (108) pole figure shows that the (108) peak is highest in the range from 12° to 36° to the shell surface normal, with a maximum at ~25°. This is exactly to be expected as the angle between the (001) and the (108) planes is 26°. Thus their observation is quite consistent with there being some crystals with the *c*-axis parallel and others with the (104) pole parallel, to the shell normal. Because the preferred orientation is not perfect, we expect a few degrees of variation in angle, so that high (108) peaks are observed all the way from a few degrees below 18° to a few degrees above 26°. None of the other published pole figures in the work is inconsistent with this conclusion.

In the work of Favejee et al., as in the present work, the x-ray beam covers a large number of crystals, so that the results obtained represent a collective behavior of the crystals. By contrast, electron diffraction methods allow the orientation of only a small number of crystals to be observed. It is impossible to draw general conclusions about the overall crystalline orientation throughout the shell when results are consolidated from only a few crystals taken from several different specimens, as was the case in the work of Perrott et al. (1981). Only 8 specimens of the surface layer were examined; the orientations of a total of only 19 crystals were determined, and a tendency for the (104) planes to lie parallel to the surface was noted. Eight chemically-thinned specimens and one ion-beam thinned specimen from the central layer were analyzed. The orientations of a total of 24 crystals from the former were determined and found to be essentially random. Results from 14 crystals in the ion-beam thinned specimen showed a small degree of preferred orientation with the *c*-axis at 30° $\pm$ 18° to the surface normal. Results collated from different columns, however, showed no preferred orientation in the central layer. In another electron diffraction

study, of the quail eggshell (Quintana and Sandoz, 1978), only one crystal from each of five neighboring columns, in the external cone layer, were analyzed. The *c*-axes of four of the crystals were oriented at ~45° to the surface normal and that of the fifth at ~60°. In other than the ratite and tinamou eggshells, very little preferred orientation may be expected to be evident at so early a stage in a shell's development; moreover, the quail eggshell has been shown to exhibit only a weak (001) preferred orientation that develops through its thickness (H. Silyn-Roberts and R. M. Sharp, 1984). Clearly, the overall pattern of crystal orientation cannot be elicited by using this method when statistically so few crystals are surveyed. Only by analysis of a comparatively large area of a shell, a material known to have exact requirements in terms of strength and total effective pore area, can the precision of its development be accurately assessed.

APPENDIX

Calculation of Depth for 99% Attenuation of Initial Beam

The absorption of a beam of radiation is given by the equation $i = i_0 \exp -(\mu/\rho)\,\rho x$, where i_0 is the initial intensity of the beam; i, the intensity after traversing through material of thickness x; μ/ρ, the mass absorption coefficient; and ρ, the density of material. The mass absorption coefficient for CoKα radiation in calcium carbonate is 12.1 m^2kg^{-1} (Birks, 1971). Density of eggshell is between 2,000 and 2,500 kg m^{-3} (Romanoff and Romanoff, 1949). Assuming a mean value for density of 2,250 kg m^{-3} and taking $i/i_0 = 0.01$, the equation above gives a path length for the radiation of 169 μm for 99% attenuation of the initial intensity. The depth of material, h, in which this attenuation takes place can easily be calculated from the geometric relationship between the path length, x, and the Bragg angle, θ, viz, $2h = x \sin\theta$. For the highest Bragg angle used in this study (28.5°), the depth is calculated to be 40 μm.

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